Journal of Medical Virology 49:289–295 (1996)

Detection of Human Herpes Virus 6 in AIDS-Associated Retinitis by Means of In Situ Hybridization, Polymerase Chain Reaction and Immunohistochemistry

Anne-Marie Fillet, Isabelle Reux, Cathy Joberty, Jean-Guy Fournier, Jean-Jacques Hauw, Phuc Le Hoang, François Bricaire, Jean-Marie Huraux, and Henri Agut

Laboratory of Bacteriology-Virology, CNRS EP 57 (A.-M.F., C.J., J.-M.H., H.A.), Ophthalmology Department (I.R., P.L.H.), Neuropathology Laboratory (J.-J.H.), and Infectious Disease Department (F.B.), Pitié-Salpêtrière Hospital, and INSERM U43 Saint-Vincent de Paul Hospital (J.-G.F.), Paris, France

The ubiquitous nature of HHV-6 and its genomic relationship with cytomegalovirus led us to evaluate an etiological link between HHV-6 and AIDS-associated retinitis in a prospective study. HHV-6 infection was studied in patients with AIDS-associated retinitis and in two control populations. Eye pairs were obtained at necropsy from nine patients with AIDS-associated retinitis, four human immunodeficiency virus (HIV)-seropositive patients with normal fundus examination and three HIV-seronegative patients. HHV-6 infection was detected by polymerase chain reaction (PCR), in situ hybridization and immunohistochemistry. Human cytomegalovirus (CMV) and HIV-1 infections were detected in parallel by the same methods.

HHV-6 infection was detected in three cases of AIDS-associated retinitis. In two of these patients, HHV-6 infection was detected both by immunohistochemistry and PCR while in the third case it was detected by in situ hybridization and PCR. In the three patients, fundus examination showed bilateral retinitis in two of them and unilateral retinitis in one of them. HHV-6 infection was not detected in the retina of the two control groups. CMV was also detected in the three cases positive for HHV-6 by all three methods. HIV DNA was detected by PCR in two of three cases and was confirmed in one of these cases by in situ hybridization. These results confirm that HHV-6 infects the retina but suggests that HHV-6 does not have an exclusive causative role in AIDS-associated retinitis, since CMV coinfection of the retina was detected in all three of the patients positive for HHV-6.

KEY WORDS: HHV-6, retinitis, HIV-1, CMV, AIDS, PCR, in situ hybridization, immunohistochemistry

INTRODUCTION

Human herpesvirus 6 (HHV-6) was isolated originally from patients with lymphoproliferative disorders and acquired immunodeficiency syndrome (AIDS) [Salahuddin et al., 1986]. As for other herpesviruses, HHV-6 infection remains in a latent state after primary infection. Blood mononuclear cells, human monocytes-macrophages [Kondo et al., 1991], salivary glands [Fox et al., 1990; Krueger et al., 1990] and lymph nodes [Fillet et al., 1995] are believed to be latency sites. Primary infection with HHV-6 in children causes Exanthema Subitum, fever, rash, and seizures [Yamanishi et al., 1988; Pruksananonda et al., 1992; Caserta et al., 1994; Breese Hall et al., 1994; Ishiguro et al., 1990; Suga et al., 1993]. McCaughey et al. [1993] reported no evidence of association between HHV-6 and retinopathy in non-immunocompromised subjects in a controlled study.

The pathogenic role of HHV-6 as an opportunistic agent has been suggested by analogy with cytomegalovirus. In fact, HHV-6 shares many common characteristics with cytomegalovirus, including DNA sequence homology, similar genomic organization [Lawrence et al., 1990] and similar sensitivity to antivirals, which has led to the classification of both viruses in the same Betaherpesvirinae subfamily. Cytomegalovirus is reactivated commonly in immunocompromised patients, causing a wide spectrum of diseases. HHV-6 is also frequently reactivated in immunocompromised patients [Cone et al., 1993]. Cytomegalovirus retinitis is observed in 15–40% of AIDS patients [Freeman et al., 1984; Holland et al., 1983; Jabs et al., 1989] but in this population, the actual etiology of some atypical AIDS-retinitis remains uncertain. This led us to evaluate an etiological link between

Accepted for publication March 18, 1996.

Address reprint requests to Anne-Marie Fillet, MD, PhD, Virology Laboratory, CERVI, Groupe Hospitalier Pitié-Salpêtrière, 47-83 Bd. de l'Hôpital, 75651 Paris Cedex 13, France.

HHV-6 and AIDS-associated retinitis. Previously, HHV-6 DNA sequences have been detected in AIDS-associated retinal lesions in one patient by PCR [Qavi et al., 1989] and HHV-6 antigens in two patients by immunohistochemistry [Reux et al., 1992]. These studies did not include control subjects, which is nevertheless a crucial requirement due to the high prevalence of HHV-6 infection in the general population.

In the present study three different approaches, PCR, in situ hybridization and immunohistochemistry, were used to detect HHV-6 in patients with either unilateral or bilateral AIDS-associated retinitis, and the findings were compared with those observed in two control populations: HIV-seronegative and HIV-seropositive subjects *with a normal* fundus. All samples were tested for the presence of CMV and HIV-1 by the same methods.

MATERIALS AND METHODS

Cases

Thirteen AIDS patients for whom ophthalmic examination had been carried out 60 days or less before death were studied. They were classified prospectively into three groups on the basis of fundus examination results (Table I). Group 1 was composed of four patients who had a normal fundus (1.1 to 1.4), group 2 of five patients who had bilateral retinitis (2.1 to 2.5) and group 3 of four patients who had unilateral retinitis (3.1 to 3.4). Three HIV-1 seronegative patients who had no retinitis at the time of death were studied by the same methods *as controls*.

Samples

In each case, eyes were removed at autopsy, frozen in isopentane cooled by liquid nitrogen and stored at −80°C. Adjacent 10 μm cryostat sections were obtained and used for histological study, in situ hybridization (ISH) and immunohistochemistry (IHC). Larger fragments were used for PCR. For histological study, tissue sections were fixed in 4% formaldehyde for 10 minutes, after which haematoxylin-eosin staining was undertaken.

Tissue DNA Extraction and Detection of HHV-6, CMV and HIV-1 Using PCR

Samples were lysed for 1 hour in Tris EDTA (10 μM Tris hydrochloride pH 7.5, 1 mM EDTA) buffer containing 0.5% sodium dodecyl sulphate and 500 μg/ml of proteinase K at 52°C. DNA was extracted by repeated phenol-chloroform treatments, ethanol-precipitated, resuspended in 200 μl water and kept frozen at −20°C.

Serial tenfold dilutions (from undiluted to 10^{-6}) of DNA samples were tested preliminarily for beta-globin gene amplification [Saiki et al., 1987] in order to detect putative PCR inhibitors and determine the effect of dilution on PCR inhibition. If beta-globin PCR was negative, another extraction was performed after which beta-globin PCR was tested again. In most cases beta-globin amplification provided a stronger signal at dilution 1:100 than at dilution 1:10 or when using undiluted samples, thus revealing the presence of inhibitors.

Three distinct HHV-6 amplification assays were used for virus detection with different primer pairs as previously described [Collandre et al., 1990; Aubin et al., 1993; Gautheret et al., 1996], one CMV amplification assay [Fillet et al., 1993] and three distinct HIV-1 amplification assays [Lauré et al., 1988; Ou et al., 1988; Defer et al., 1992]. Ten microliters of each DNA dilution were amplified and examined essentially as described previously [Fillet et al., 1995].

In Situ Hybridization

In situ hybridization was undertaken essentially as described previously for HIV-1 [Reux et al., 1993] and for CMV [Reux et al., submitted 1996]. As a HHV-6 probe, we used the plasmid pHC 44 containing the 3.6 kbp-long ClaI fragment of HHV-6 genome (SIE strain) inserted in pBR 322 plasmid. The HHV-6 probe was labelled by nick-translation with α-[^{35}S]-dATP (Amersham) to a specific activity of 1.7 10^8 cpm/μg.

Immunohistochemistry

Immunohistochemistry was performed as previously described for HIV-1 [Reux et al., 1993] and for CMV [Reux et al., submitted 1996]. The monoclonal HHV-6 antibody OHV1 [Okuno et al., 1990] at 1:500 dilution was applied overnight.

RESULTS

Histological Study

In group 1 patients without fundus examination abnormality, histological examination was normal in three cases (1.1, 1.3, 1.4) and showed typical cytomegalic cells in case 1.2 (Table II). There were typical CMV retinitis lesions concordant with the ophthalmological results for all group 2 patients in whom bilateral retinitis had been found. CMV retinitis was diagnosed on the basis of focal changes with disorganization of the retinal architecture, mononuclear and polymorphonuclear cell infiltrates, particularly around blood vessels. Several cells had large intranuclear and intracytoplasmic inclusions suggestive of CMV infection. For group 3 patients with unilateral retinitis, histological retinitis lesions were observed in the case of typical CMV retinitis at fundus examination as well as in the case of cotton-wool spots. Histological lesion of CMV retinitis was not observed for the eye with normal fundus aspect in the case of subject 3.3.

HHV-6 Detection

HHV-6 DNA was detected using PCR in three cases of AIDS-associated-retinitis (2.1, 2.4, 3.2) (Table II). In these three cases, the positive result was reproducibly obtained only when testing 100-fold diluted DNA: it was not obtained either with further DNA dilutions or with pure and 10-fold diluted DNA. HHV-6 infection was confirmed by in situ hybridization in the case of subject 2.4. HHV-6 signal was detected in very few cells of neurosensorial retina, located mainly in ganglion cell layer, within retinitis lesion. Hybridization signals were more prominent in cytoplasm than in nucleus (Fig. 1A). Some HHV-6 positive cells exhibited cytopathic traits such as swollen

TABLE I. Clinical Disorders and Ophthalmologic Features in the Three Groups of AIDS Patients

Group	Subject	Age	Systemic disorders	Fundus examination	Delay between fundus examination and death (days)
1	1.1	34	Mucocutaneous candidiasis meningoencephalitis, cryptosporidiosis	N	37
	1.2	32	Meningoencephalitis, frontal and cerebellar tumor	N	9
	1.3	24	Mucocutaneous candidiasis, cryptosporidiosis, pulmonar tuberculosis	N	37
	1.4	29	Progressive multifocal leukoencephalopathy	N	30
2	2.1	34	Cerebral toxoplasmosis, pneumocystis carinii pneumonia, CMV sclerosing cholangitis, salmonella sepsis	Bilateral CMV retinitis	30
	2.2	47	Mucocutaneous candidiasis, cytolytic hepatitis, pneumonia	Bilateral CMV retinitis	30
	2.3	33	Mucocutaneous candidiasis, pneumocystis carinii and CMV pneumonia	Bilateral CMV retinitis	60
	2.4	44	Cerebral toxoplasmosis, mucocutaneous candidiasis, CMV pneumonia, peripheral neuropathy	Bilateral CMV retinitis	11
	2.5	24	Pneumocystis carinii pneumonia, CMV colitis, mycobacterium avium intracellulare septicemia, cerebral toxoplasmosis and CMV encephalitis	Bilateral CMV retinitis	17
3	3.1	35	Cutaneous Kaposi's sarcoma, pulmonar tuberculosis, mucocutaneous HSV and candidiasis, colitis	Unilateral CMV retinitis (Cotton-wool spots in opposite eye)	40
	3.2	28	Mucocutaneous candidiasis, pneumocystis carinii and CMV pneumonia	Unilateral CMV retinitis (Cotton-wool spots in opposite eye)	3
	3.3	32	Mucocutaneous candidiasis, pneumocystis carinii pneumonia, abdominal zoster, salmonella septicemia	Normal (CMV retinitis in opposite eye)	12
	3.4	30	Staphylococcus endocarditis, mucocutaneous HSV, pulmonar tuberculosis	Unilateral CMV retinitis (Cotton-wool spots in opposite eye)	45

CMV, cytomegalovirus; HSV, herpes simplex virus; N, normal.

cells (Fig. 1B). Using immunohistochemistry, HHV-6 antigen was detected in the case of subjects 2.1 and 3.2. As previously published [Reux et al., 1992] both cytoplasmic and nuclear HHV-6 positive granulations, both small and large, were observed. HHV-6 positive cells were mainly located in the ganglion cell layer and in the inner nuclear cell layer. HHV-6 was not detected in the retina of either group 1 subjects or HIV-1-seronegative control subjects.

CMV and HIV-1 Detection

CMV was detected in the three patients 2.1, 2.4 and 3.2 concomitantly with the detection of HHV-6 (Table II). In the three patients, CMV was detected concomitantly by all the methods used. CMV-positive cells were found at high frequency within retinitis area, and their number was higher than that of HHV-6-positive cells. By comparing the results obtained on adjacent retina sections, the HHV-6-positive cells were seen to be located within the area containing CMV-positive cells. Some cells provided a positive signal on adjacent sections both for CMV and for HHV-6, suggesting that they were co-infected by the two viruses (not shown). In parallel with the detection of HHV-6, HIV-1 DNA was detected by PCR in the cases 2.1 and 2.4. In the case of the subject 2.1, this positive result was confirmed using in situ hybridization [Reux et al., 1993]. HIV-1 p24 antigen was not detected in the retina cells in any of the three cases positive for HHV-6.

DISCUSSION

PCR and in situ analysis including both in situ hybridization and immunohistochemistry revealed that three of the nine patients with AIDS-associated retinitis stud-

TABLE II. Histological Findings and Results of HHV-6, CMV and HIV-1 Detection in the Retina of HIV-1 Infected Patients

		Virus detected				
		HHV-6				
Subject	Histological findings	PCR	In situ hybridization	Immunohistochemistry	CMV[a]	HIV-1[b]
1.1	Normal	–	NT	NT	+	+
1.2	Large cells in retina with viral inclusions, CMV retinitis	–	–	–	+	+
1.3	Normal	–	NT	–	–	–
1.4	Normal	–	–	–	+	–
2.1	Large cells in retina with viral inclusions, CMV retinitis	+	NT	+	+	+
2.2	Large cells in retina with viral inclusions, CMV retinitis	–	–	–	+	+
2.3	Retinal necrosis, cytomegalic cells in neurosensorial retina and pigment epithelium, CMV retinitis	–	–	IC	+	+
2.4	Large cells in retina with viral inclusions, CMV retinitis	+	+	–	+	+
2.5	Large cells in retina with viral inclusions, CMV retinitis	–	NT	–	+	+
3.1	Left eye: large cells in retina with viral inclusions, CMV retinitis	–	–	–	+	–
3.2	Right eye: large cells in retina with viral inclusions, CMV retinitis	+	NT	+	+	–
3.3	Normal for eye with normal fundus aspect	–	NT	–	+	+
3.4	Left eye: large cells in retina with viral inclusions, CMV retinitis	–	NT	–	+	+

[a]Morphological results are presented in detail elsewhere [Reux et al., 1996].
[b]Morphological results are presented in detail elsewhere [Reux et al., 1993].
NT, not tested; IC, inconclusive; +, positive; –, negative.

ied had HHV-6-infected cells in the retina. The HHV-6-infected cells were few, essentially located in the ganglion cell layer and present in areas of histologically defined retinitis. The detection of a late HHV-6 antigen by immunohistochemistry (subjects 2.1 and 3.2) proved that HHV-6 was present in a replicative state and was not latent. The concomitant detection of HHV-6 RNA and DNA by in situ hybridization (subject 2.4) also provided circumstantial evidence for active expression of HHV-6 genome in infected cells. These results are in agreement with the transcriptional activity of HHV-6 described in lesions located in the optic nerve area and the retinal layers from two AIDS patients [Qavi et al., 1992]. In contrast, HHV-6 infection was not found in HIV-positive and HIV-negative subjects without retinitis, suggesting HHV-6 infection was specifically associated with retinitis. In this sense, our results are different from those reported by Qavi et al. [1992], who observed HHV-6 antigens not only in AIDS-associated retinitis but also in the retina of HIV-infected patients with no visible lesions on fundus examination [Qavi et al., 1994]. Such discrepancy could be explained by several reasons. The differences in the detection of HHV-6 antigens could be due to the use of different monoclonal antibodies. We used OHV-1 antibody which detects a late antigen, is very sensitive and reacts well with the two HHV-6 variants [Okuno et al., 1992]. The properties of OHV-1 antibody should be compared to those of the monoclonal antibody used by Qavi et al. [1994], for which more information is needed. Alternatively, these discordant results may also be explained by a higher number of HHV-6-infected cells in retinitis than in the normal retina of AIDS patients. As a contribution to this debate, it is worth noting that in a study of 50 AIDS patients with retinal disease, HHV-6 DNA was not detected in any of the vitreous samples whereas CMV was found [Mitchell et al., 1994]. This result could be explained by a higher number of CMV-infected than HHV-6-infected cells in retinitis, as observed in the present study. We could not exclude HHV-6 infection of the normal retina of AIDS patients at a very low frequency explaining the negative results obtained in the control samples.

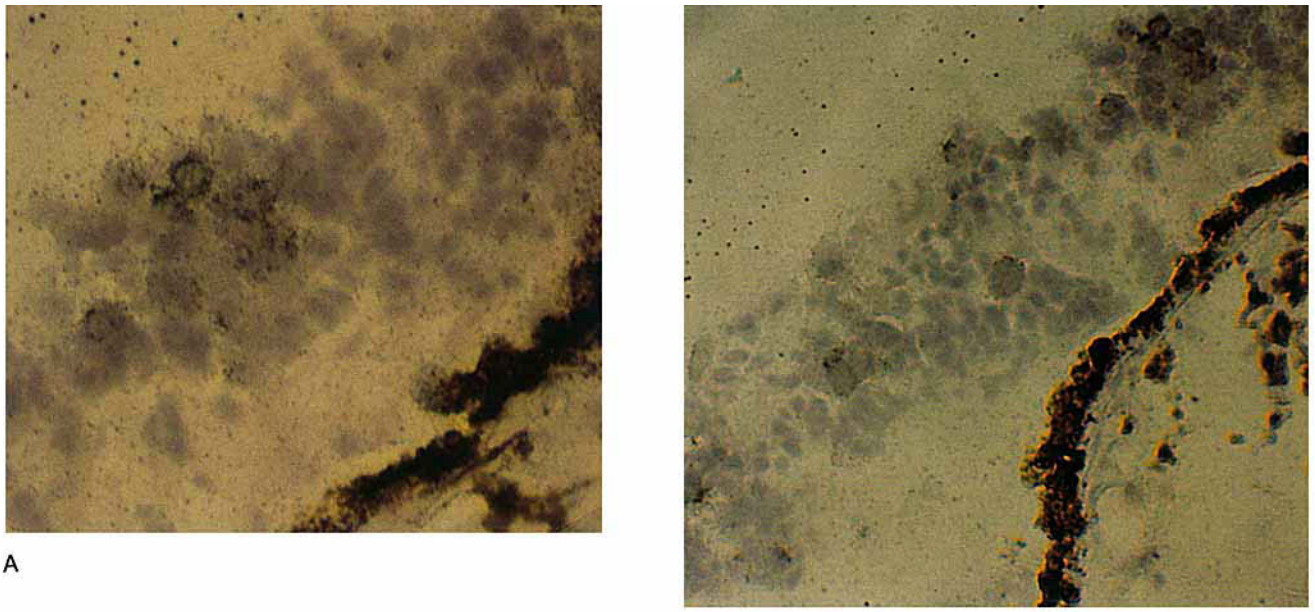

Fig. 1. Detection of HHV-6 infection in AIDS retinitis by means of in situ hybridization. The strong brown layer is the retinal pigment epithelium. **A:** Some cells of neurosensorial retina contain HHV-6 DNA and RNA. **B:** Some HHV-6-infected cells of neurosensorial retina are swollen cells.

To detect HHV-6 infection in retina tissues, we used in parallel PCR on DNA extracts and in situ detection of either antigen or DNA and RNA on tissue sections. Surprisingly, cumulative results of in situ detection were identical to those of PCR, indicating that both procedures exhibited the same sensitivity. PCR is theoretically more sensitive than morphological methods such as immunohistochemistry and in situ hybridization for detecting viral infection. In fact, when performed on retina samples, PCR was found to be less sensitive than for other tissues [Fillet et al., 1995]. Several reasons may explain this lack of sensitivity. The presence of PCR inhibitors in DNA extracted from retinal tissue was demonstrated by the inhibition of beta-globin gene PCR in some cases. These inhibitors might be pigment products derived from retinal pigment epithelium. The presence of these inhibitors required both repeated DNA extractions and template dilution in PCR assays. Both procedures were efficient: repeated extractions improved the ability of DNA to be amplified and diluted DNA often provided stronger PCR signals than undiluted samples, as assessed by beta-globin gene PCR. Unfortunately, these two necessary procedures decreased considerably the sensitivity of detection.

The concomitant presence of at least three viruses, HHV-6, CMV and HIV-1, in retinal tissue raises the question of their respective causative roles and their mutual interactions in AIDS retinitis. CMV appeared to be present in a greater number of cells and at a higher level than HHV-6 in retinitis lesions. Although it was also detected in retinal tissue with no obvious retinitis lesions, CMV was probably the main pathogenic virus for the cases studied. HIV-1 DNA was detected in two of the three cases by PCR. In situ analysis previously indicated that HIV-1 was located in retinal vascular walls [Pomerantz et al., 1987; Reux et al., 1993]. However, in the cases studied, HIV-1 was located outside the retinitis area, which did not support the hypothesis of a direct role of HIV-1 in the genesis of retinitis lesions. HHV-6 was detected specifically in retinitis lesions but at a lower frequency and with fewer infected cells than CMV. Furthermore, HHV-6 infection was associated with CMV infection in the three cases we studied as in the two cases described by Qavi et al. [1992]. Thus, the putative role of HHV-6 as an agent of AIDS retinitis could not be demonstrated unambiguously from our data. It is likely that the close association between HHV-6 and CMV will be a major obstacle for the clear demonstration of the respective role of both viruses. Alternatively, the close association between both viruses might be considered a major determinant for their pathogenicity in retina. Recently in vitro studies have shown that expression of HHV-6 genes can interact with that of CMV genes when they both occur in the same cell: a protein called B115 has been shown to transactivate immediate early gene CMV promoter [Razzaque and Jones, 1995]; gL HHV-6 glycoprotein can form heterodimer complexes with gH CMV glycoprotein [Anderson et al., 1995]. According to our preliminary findings, mixed infection with HHV-6 and CMV probably occurs in retina cells and synergy in gene expression might occur between the two viruses. In addition, as for other herpesviruses, HHV-6 infection induces local alterations of cytokine expression and therefore modulates CMV expression in neighboring cells [Flamand et al., 1991, 1995]. The location of both HHV-6 and CMV-infected

cells in the same areas of retinitis lesions fits with this hypothesis. Ultimately, the association between HHV-6 and CMV raises some concern about the treatment of AIDS retinitis. In in vitro susceptibility assays, HHV-6 appeared to be as sensitive to foscarnet and ganciclovir as CMV [Agut et al., 1989]. However, the possible synergy between the two viruses might modify the conditions of inhibition of their DNA polymerases and alter their susceptibility to antivirals. This question obviously requires further study.

ACKNOWLEDGMENTS

We thank Marie-Christine Papuchon and Sylvie Pastol for manuscript typing, Koichi Yamanishi for the gift of HHV-6 monoclonal antibody OHV-1, Hélène Collandre for the gift of HHV-6 plasmid pHC44 and Dorian McIlroy for English revision. Supported by the Agence Nationale de Recherche sur le SIDA grant 89060 and the National Institute of Health grant EYO 837.

REFERENCES

Agut H, Collandre H, Aubin JT, Guétard D, Favier V, Ingrand D, Montagnier L, Huraux JM (1989): In vitro sensitivity of human herpesvirus 6 to antiviral drugs. Research in Virology 140: 219–228.

Anderson RA, Liu DX, Macaulay HA, Gompels UA (1995): Definition of a betaherpesvirus specific gH domain required for gH/gL complex formation. 127. The XXth International Herpesvirus Workshop, July 29–August 3. Groningen, The Netherlands.

Aubin JT, Agut H, Collandre H, Yamanishi K, Chandran B, Montagnier L, Huraux JM (1993): Antigenic and genetic differentiation of the two putative types of human herpesvirus 6. Journal of Virological Methods 41:223–234.

Breese Hall C, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG (1994): Human herpesvirus 6 infection in children. New England Journal of Medicine 331(7):432–438.

Caserta MT, Hall CB, Schnabel K, McIntyre K, Long C, Costanzo M, Dewhurst S, Insel R, Epstein LG (1994): Neuroinvasion and persistence of human herpesvirus 6 in children. Journal of Infectious Diseases 170:1586–1589.

Collandre H, Aubin JT, Agut H, Bechet JM, Montagnier L (1990): Detection of HHV-6 by the polymerase chain reaction. Journal of Virological Methods 31:171–180.

Cone RW, Hackman RC, Huang M-LW, Bowden RA, Meyers JD, Hetcalf M, Zeh J, Ashley R, Corey L (1993): Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. New England Journal of Medicine 329:159–161.

Defer C, Agut H, Garbarg-Chenon A, Moncany M, Morinet F, Vignon D, Mariotti M, Lefrère JJ (1992): Multicentric quality control of polymerase chain reaction for detection of HIV DNA. AIDS 6: 659–663.

Fillet AM, Katlama C, Visse B, Camilleri S, Rogeaux O, Huraux JM (1993): Human CMV infection of the CNS: Concordance between PCR detection in CSF fluid and pathological examination. AIDS 7 (7):1016–1018.

Fillet AM, Raphael M, Visse B, Audouin J, Poirel L, Agut H, and French study group for HIV associated tumors (1995): A controlled study of human herpesvirus 6 detection in AIDS-associated non Hodgkin's lymphoma. Journal of Medical Virology 45:106–112.

Flamand L, Gosselin J, D'Addario M, Hiscott J, Ablashi DV, Gallo RC, Menezes J (1991): Human herpesvirus 6 induces interleukin-1 β and tumor necrosis factor alpha, but not interleukin-6, in peripheral blood mononuclear cell cultures. Journal of Virology 65: 5105–5110.

Flamand L, Gosselin J, Stefanescu I, Ablashi D, Menezes J (1995): Immunosuppressive effect of human herpesvirus 6 on T-cell functions: Suppression of interleukin-2 synthesis and cell proliferation. Blood 85:1263–1271.

Fox JD, Briggs M, Ward PA, Tedder RS (1990): Human herpesvirus 6 in salivary glands. Lancet 336:590–593.

Freeman WR, Lerner CW, Mines JA, Lash RS, Nadel AJ, Starr MB, Tapper ML (1984): A prospective study of ophthalmologic findings in the acquired immune deficiency syndrome. American Journal of Ophthalmology 97:133.

Gautheret A, Aubin JT, Poirel L, Chopineau S, Agut H, Dussaix E (1996): Detection and variant identification of HHV-6 by a non radioactive hybridization microplate assay for amplimers detection. Journal of Virological Methods 58:33–40.

Holland GN, Pepose JS, Pettis TH, Gottlieb MS, Yee RD, Foos RY (1983): Acquired immunodeficiency syndrome. Ophthalmology 90: 859.

Ishiguro N, Yamada S, Takahashi T, Takahashi Y, Togashi T, Okuno T, Yamanishi K (1990): Meningoencephalitis associated with HHV-6 related exanthem subitum. Acta Paediatrica Scandinavica 79: 987–989.

Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K (1991): Latent human herpes virus 6 infection of human monocytes/macrophages. Journal of General Virology 72:1401–1408.

Krueger GRF, Wassermann K, De Clerck LS, Stevens WJ, Bourgeois N, Ablashi DV, Josephs SF, Balachandran N (1990): Latent herpesvirus-6 in salivary and bronchial glands. Lancet 336:1255–1256.

Jabs DA, Green WR, Fox R, Polk BF, Bartlett JG (1989): Ocular manifestations of acquired immunodeficiency syndrome. Ophthalmology 96:1092.

Laure F, Rouzioux C, Veber F, Jacomet C, Courgnaud V, Blanche S, Burgard M, Griscelli C, Brechot C (1988): Detection of HIV-1 DNA in infants and children by means of the polymerase chain reaction. Lancet ii:538.

Lawrence GL, Chee M, Craxton MA, Gompels UA, Honess RW, Barrell BG (1990): Human herpesvirus 6 is closely related to human cytomegalovirus. Journal of Virology 64:287–299.

McCaughey C, Gormley P, Connoly JH, Archer DB, Coyle PV (1993): Antibodies to rubella and human herpesvirus 6 in diverse retinal diseases. Journal of Infectious Diseases 167:1499–1500.

Mitchell SM, Fox JD, Tedder RS, Gazzard BG, Lightman S (1994): Vitreous fluid sampling and viral genome detection for the diagnosis of viral retinitis in patients with AIDS. Journal of Medical Virology 43:336–340.

Okuno T, Higashi K, Shiraki K, Yamanishi K, Takahashi M, Kokado Y, Ishibashi I, Takahara S, Sonoda T, Tanaka K, Baba K, Yabuuchi H, Kurata T (1990): Human herpesvirus 6 in renal transplantation. Transplantation 49:519–522.

Okuno T, Shao H, Asada H, Shiraki K, Takahashi M, Yamanishi K (1992): Analysis of human herpesvirus 6 glycoproteins recognized by monoclonal antibody OHV1. Journal of General Virology 73: 443–447.

Ou CH, Kwok S, Mitchell SW, Mack DH, Snisky JJ, Krebs JW, Feorino P, Warfield D, Schochetman G (1988): DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science 239:295–297.

Pomerantz PJ, Kwutzkes DR, De la Monte SM, Rota JR, Bauer AS, Albert D, Bor DH, Feldman EL, Schoolez RT, Hirsch MS (1987): Infection of the retina by immunodeficiency virus type I. New England Journal of Medicine 317:1643–1647.

Pruksananonda P, Hall CB, Insel RA, McIntyre K, Pelett PE, Long CE, Schnabel KC, Pincus PH, Stamey FR, Dambaugh TR, Stewart JA (1992): Primary human herpesvirus 6 infections in young children. New England Journal of Medicine 326:1445–1450.

Qavi HB, Green MT, Segall GK, Font RL (1989): Demonstration of HIV-1 and HHV-6 in AIDS-associated retinitis. Current Eye Research 8:379–387.

Qavi HB, Green MT, Segall GK, Lewis DE, Hollinger FB (1992): Transcriptional activity of HIV-1 and HHV-6 in retinal lesions from AIDS patients. Investigative Ophthalmology Visual Science 33: 2759–2767.

Qavi HB, Green MT, Lewis DE, Hollinger FB, Pearson G, Ablashi D (1994): Implication of HIV-1 and HHV-6 in the development of AIDS retinitis. (333B) Tenth International Conference on AIDS. Yokohama, Japan, 7–12 August.

Razzaque A, Jones C (1995): Characterization of a transactivator protein identified in the transforming domain ZVH14 of human herpesvirus-6: Activation of heterologous promoters including the H-RAS. 182. The XXth International Herpesvirus Workshop, July 29–August 3. Groningen, The Netherlands.

Reux I, Fillet AM, Agut H, Katlama C, Le Hoang P, Hauw JJ (1992): In situ detection of human herpes virus 6 in AIDS associated retinitis. American Journal of Ophthalmology 114(3):375–377.

Reux I, Fillet AM, Fournier JG, Agut H, Gentilini M, Le Hoang P,

Hauw JJ (1993): In situ hybridization of HIV-1 RNA in retinal vascular wall. American Journal of Pathology 143:1275–1279.

Reux I, Fillet AM, Fournier JG, Joberty C, Agut H, Katlama C, LeHoang P, Hauw JJ (1996): Cytomegalovirus (CMV) and human immunodeficiency virus (HIV-1) detection in the retina. A controlled clinicopathological study using immunohistochemistry, in situ hybridization and DNA amplification. Current Eye Research (submitted).

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1987): Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenzgger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC (1986): Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234:596–600.

Suga S, Yoshikawa T, Asano Y, Kozawa T, Nakashima T, Kobayashi I, Yazaki T, Yamamoto H, Kajita Y, Ozaki T, Nishimura Y, Yamanaka T, Yamada A, Imanishi J (1993): Clinical and virological analyses of 21 infants with exanthem subitum and central nervous system complications. Annals of Neurology 33(6):597–603.

Yamanishi K, Shiraki K, Kondo T, Okuno T, Takahashi M, Asano Y, Kurata T (1988): Identification of human herpesvirus 6 as causal agent for exanthem subitum. Lancet i:1065–1067.